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L9: Entry 4 of 13 File: USPT Dec 28, 1999

DOCUMENT-IDENTIFIER: US 6007987 A
TITLE: Positional sequencing by hybridization

DEPR:
Preparation of model arrays. Following the scheme shown in FIG. 2, in a single synthesis, all 1024 possible single-stranded probes with a constant 18 base stalk followed by a variable S base extension can be created. The 18 base extension is designed to contain two restriction enzyme cutting sites. Hga I generates a 5 base, 5' overhang consisting of the variable bases N.sub.5. Not I generates a 4 base, 5' overhang at the constant end of the oligonucleotide. The synthetic 23-mer mixture will be hybridized with a complimentary 18-mer to form a duplex which can then be enzymatically extended to form all 1024, 23-mer duplexes. These can be cloned by, for example, blunt end ligation, into a plasmid which lacks Not I sites. Colonies containing the cloned 23-base insert can be selected. Each should be a clone of one unique sequence. DNA minipreps can be cut at the constant end of the stalk, filled in with biotinylated pyrimidines, then cut at the variable end of the stalk, to generate the 5 base 5' overhang. The resulting nucleic acid can be fractionated by Qiagen columns (nucleic acid purification columns) to discard the high molecular weight material, and the nucleic acid probe will then be attached to a streptavidin-coated surface. This procedure could easily be automated in a Beckman Biomec or equivalent chemical robot to produce many identical arrays of probes.

L9: Entry 1 of 13 File: USPT Sep 18, 2001

DOCUMENT-IDENTIFIER: US 6291181 B1
TITLE: Nucleic acid adapters containing a type IIs restriction site and methods of using the same

DEPR:
In the case of high molecular weight nucleic acids, the original polynucleotide sequence will generally comprise more than one and even several specific Type-IIs endonuclease recognition/cleavage sites, e.g., EarI sites. As a result, a number of ambiguous sequence segments will be captured for a given polynucleotide. Upon probing with an oligonucleotide array, the sequence will hybridize with a number of probes which are complementary to all of the captured sequences, producing a distinctive hybridization pattern for the given polynucleotide sequence. The specific hybridization pattern of the target sequence upon the array will generally indicate the ambiguous sequences adjacent to all of the cleavage sites as was described above.